30. (New) The method of claim 23 wherein the first agent is 5-aza-2' deoxycytidine, the second agent is trichostatin A, and the third agent is Tat-cyclin B.

31. (New) The method of claim 30 comprising treating the reprogrammed keratinocyte with retinoic acid, wherein the reprogrammed keratinocyte after treating with the retinoic acid expresses a gene product selected from the group consisting of neurofilament, cardiac actin and alpha-antitrypsin.

C3 Amend

32. (New) The method of claim 31 wherein the gene product is a human cardiac actin RNA.

33. (New) The method of claim 30 wherein the keratinocyte is a human keratinocyte.

Remarks

Rejections under 35 USC § 112, first paragraph

Claims 1-9 are rejected as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. Examiner contends "the claims are broadly drawn to reprogramming any human somatic cell into a pluripotent cell by administering to said cell an agent which promotes cellular reprogramming." The stated basis of this rejection is the alleged "lack of specific guidance necessary to practice the instantly claimed method" and the "simplistic solution of applying an [single] agent to a cell to reprogram a cell to become a totipotent stem cell ... is not supported in the art nor the specification." Several papers are cited by Examiner which describe the complex cell biology associated with changes in either methylation or acetylation. Applicants acknowledge that cellular reprogramming is complex, as depicting in the literature (Kikyo *et al.*, Walsh *et al.* and Keohane *et al.*) However, the facts presented in the actual working examples (Examples 2, 3, 4 pages 30-34 of the specification) demonstrate that an adult somatic cell has been reprogrammed to express a stem cell marker and to have the capability to express markers of all three primordial germ layers. The disclosure of actual working examples in this patent application makes the instant invention more than a germ of an idea.

Claims 1, 3 and 4 have been amended and claims 2, 6, 7 and 9 have been cancelled without disclaimer. Claim 1 has been amended to contain those treatment steps, which have been demonstrated by the working examples disclosed in the instant specification to sufficiently reprogram a somatic cell to express telomerase, a gene product that is known in the art to be associated with a stem cell. Please note that amended claim 1 contains elements imported from claim 2, except that, wherein claim 2 originally stated "the agent *promotes* ... the deacetylation of histone proteins," claim 1 contains the element "an agent that *inhibits* the deacetylation of histone proteins." This choice of the language used in claim 2 as originally filed with the specification was inadvertent, given that the specification expressly teaches that "somatic cells are reprogrammed via inhibition of or reversal of histone deacetylation," (page 19, lines 2, 3). Furthermore , it is well known in the art that trichostatin A, which was employed in the instant working example, inhibits histone deacetylation (see at least Cong and Bacchetti, 2000 [IDA AW]).) Thus, this amendment, which appears to be a reversal of a particular element of the original set of claims, does not contain new matter and more particularly points out and distinctly claims the invention that is described in the specification.

According to Example 2, which is an actual working example (starting at page 30 of the specification), human somatic cells (e.g., keratinocytes) were treated in vitro with an agent known in the art to promote the demethylation, (e.g., 5-aza-2' deoxycytidine), an agent known in the art to inhibit the deacetylation of histones (e.g., trichostatin A), and an agent which arrests cells in metaphase (e.g., Tat-cyclin B). After this treatment ("treatment regimen 2", page 15, lines 5 and 6; page 34, lines 2, 4; Figure 2), it was demonstrated that those treated cells express telomerase RNA, wherein the untreated cells did not express telomerase RNA (see Figure 2; page 34, lines 8-12.) Applicants assert that telomerase expression, which is "considered a hallmark gene for [an] undifferentiated cell and a requirement for sustained proliferation of a cell" (detailed action of paper no. 14, page 6, lines 18, 19), provides the skilled artisan with the reasonable expectation that the somatic cells, which have been subjected to the instant "treatment regimen 2" are stem cells.

Furthermore, those human somatic cells that have been subjected to treatment regimen 2, upon subsequent treatment with retinoic acid (which was provided merely as a method to induce differentiation of those cells to determine their pluripotency) decreased expression of telomerase RNA and significantly increased the expression of RNAs that are associated with cells derived from all three primordial germ layers (e.g., ectodermal neurofilament, mesodermal cardiac actin and endodermal alpha anti-trypsin; see working example, page 32, lines 10-21). That data provides the skilled artisan with the reasonable expectation that the somatic cells, which have been subjected to the instant "treatment regimen 2" are pluripotent cells.

Taken together, the skilled artisan would reasonably expect the somatic cells, which are treated with an agent that promotes demethylation of DNA, an agent that inhibits deacetylation of histone proteins and an agent that arrests cells in metaphase, are pluripotent stem cells. Thus, in view of the arguments and amendments presented above, Applicants request that the written description/enablement rejection of claims 1-9 under 35 USC 112, first paragraph be withdrawn.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1–9 stand rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Examiner contends that "there is no link between the reprogramming and the alteration of a somatic cell to a pluripotent cell." As described in the preceding sections, claim 1 has been amended to comprise treating a somatic cell with at least three agents (no longer at least a single agent), which is clearly supported in the actual working examples. Claim 1 has also been amended to include the element that the adult somatic cell, subsequent to the reprogramming treatment, is a pluripotent stem cell that expresses telomerase. Support for this element can be found at least on page 34, lines 8-14, which describes the expression of telomerase RNA by a somatic cell that had been treated by three agents. Claim 4 has been amended to remove the "further comprising" language. Claim 9 has been cancelled without prejudice or disclaimer. In view of the amendments to claims 1 and 4, and the cancellation of claim 9, Applicants believe the claims particularly point out and distinctly claim the subject of the instant invention. Applicants therefore request the

withdrawal of the rejection of the claims under the second paragraph of 35 USC Section 112.

Rejection under 35 U.S.C. § 102(b)

Claims 1-4 and 9 stand rejected under 35 USC Section 102(b) as allegedly being anticipated by Yoshihiko et al. (IDS reference BB). Yoshihiko teaches the treatment of gastric cancer cells with 5-aza-2' deoxycytidine, which results in the ectopic expression ABO genes. As discussed in the previous sections, claim 1 has been amended to comprise treatment of somatic cells with at least three distinct agents, one of which promotes demethylation, as does 5-aza-2' deoxycytidine. Since Yoshihiko teaches the administration of a *single* agent that reprograms a *cancer* cell in a limited way, that reference fails to teach the treating of a *somatic* cell with the novel combination of a first agent that promotes demethylation, a second agent that inhibits deacetylation and a third agent that promotes arrest at mitosis. Thus, Yoshihiko fails to teach every element of the instant claims. Applicants request that the rejection of claim 1-4 and 9 under 35 USC Section 102(b) be withdrawn.

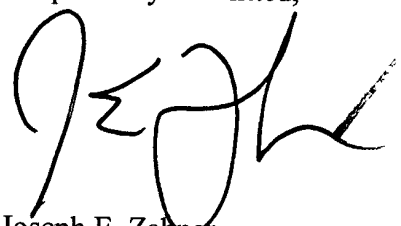
New claims introduced

New claims 21-31 have been introduced. These claims are directed to methods of producing reprogrammed cells using the steps previously presented in claims 1-9. Applicants assert that the claims, which are fully supported by the specification-as-filed and do not contain new matter, fall within the elected invention of group I. Applicants respectfully request that these claims be entered into the present case.

Conclusion

In view of the amendments and arguments set forth in this response to the Office Action of paper no. 14, Applicants believe that the claims are in condition for allowance. Applicants respectfully request that the rejection of the claims be withdrawn and the claims be allowed to issue. If any outstanding issues remain, Examiner is invited to call undersigned Applicant at the number provided to facilitate the efficient prosecution of this case.

Respectfully submitted,



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Marked–up copy of the claims (09/919,298)

1. (Amended) A method of producing a human pluripotent stem cell [cells] from a human adult somatic cell [cells] comprising treating said adult somatic cell with a first agent that promotes demethylation of nucleic acids, a second agent that inhibits the deacetylation of histone proteins, and a third agent that promotes the arrest of cells in metaphase, wherein the adult somatic cell, subsequent to treating with the first agent, the second agent and the third agent, is a pluripotent stem cell which expresses a telomerase gene product [cells with an agent that promotes cellular reprogramming].

3. (Amended) The method of claim 1 [2]wherein the first agent is [selected from the list consisting of] 5-aza-2'-deoxycytidine[, trichostatin A, a nucleoplasmin and a G2/M cyclin].

4. (Amended) The method of claim 1 [3 further] comprising treating said adult somatic cell [cells] with 5-aza-2'-deoxycytidine, trichostatin A and Tat-cyclin B.

5. The method of claim 1 wherein the adult somatic cell is a keratinocyte.

8. The method of claim 4 wherein the adult somatic cell is a keratinocyte.

21. (New) The method of claim 1 wherein the second agent is trichostatin A.

22. (New) The method of claim 1 wherein the third agent is Tat-cyclin B.

23. (New) A method of producing a reprogrammed keratinocyte comprising treating a keratinocyte in vitro with a first agent that promotes demethylation of nucleic acids, a second agent that inhibits deacetylation of histones and a third agent that promotes the arrest of mammalian cells in metaphase; wherein the reprogrammed keratinocyte expresses a telomerase gene product and is capable of expressing a gene product selected from the group consisting of neurofilament, cardiac actin and alpha-antitrypsin.

24. (New) The method of claim 23 wherein the first agent is 5-aza-2' deoxycytidine.

25. (New) The method of claim 23 wherein the second agent is selected from the group consisting of trichostatin A and sodium butyrate.

26. (New) The method of claim 25 wherein the second agent is trichostatin A.

27. (New) The method of claim 23 wherein the third agent is selecting from the group consisting of Tat-cyclin B, cyclin-A, cyclin-B, c-Mos, colchicine, and colcemid.

28. (New) The method of claim 27 wherein the third agent is Tat-cyclin B.

29. (New) The method of claim 23 wherein the keratinocyte is a human keratinocyte.

30. (New) The method of claim 23 wherein the first agent is 5-aza-2' deoxycytidine, the second agent is trichostatin A, and the third agent is Tat-cyclin B.

31. (New) The method of claim 30 comprising treating the reprogrammed keratinocyte with retinoic acid, wherein the reprogrammed keratinocyte after treating with the retinoic acid expresses a gene product selected from the group consisting of neurofilament, cardiac actin and alpha-antitrypsin.

32. (New) The method of claim 31 wherein the gene product is a human cardiac actin RNA.

33. (New) The method of claim 30 wherein the keratinocyte is a human keratinocyte.